

FIG. 6 is a bar graph showing dye signals for replicate PCR assays of various concentrations of both hCMV DNA and HIV-DNA, as described in Example 2 below.

FIG. 7 is a bar graph showing dye signals for replicate PCR assays of various concentrations of HIV-I DNA, as described in Example 3 below.

FIG. 8 is a bar graph showing dye signals for replicate PCR assays of various concentrations of HIV-I DNA, as described in Example 3 below.

FIG. 9 is a bar graph showing dye signals for replicated PCR assays of various concentrations of hCMV DNA, as described in Example 5 below.

FIG. 10 is a bar graph showing dye signals for replicated PCR assays of various concentrations of hCMV DNA, as described in Example 5 below. --

IN THE CLAIMS:

Claims as pending after entry of this amendment:

28. The method of claim 33 wherein three or more target DNA's are amplified using a set of primers for each of said target DNA's, the primers in each of said primer sets having a T_m within the range of 65 to 74°C, all of said primer T_m 's being within about 5°C of each other, and said primers in each primer set having nucleotide lengths which differ from each other by no more than 5 nucleotides.

29. The method of claim 28 wherein each of said amplified target DNA's is captured with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to

a nucleic acid sequence of a strand of a distinct amplified target DNA strand, each capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said distinct amplified target DNA strand at a temperature in the range of from 40 to 55°C.

33. A method for the simultaneous amplification and subsequent simultaneous detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a T_m within the range of from 65 to 74°C, as calculated by the formula $T_m(^{\circ}\text{C}) = 67.5 + 0.34 (\% \text{ G} + \text{ C}) - 395/\text{N}$, all of

said primer T_m 's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, each of priming and primer extension are carried out at a temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

36. A method for the simultaneous amplification and subsequent simultaneous detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated

from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides, each of said first, second, third and fourth primers having a T_m within the range of from 67 to 74°C, all of said primer T_m 's being within about 2°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i), to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands, provided that in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from 62 to 75°C,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.